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PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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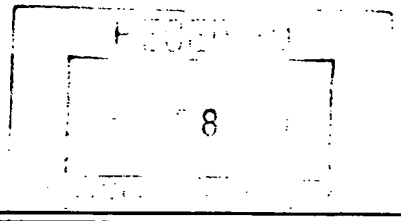

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	25 FEB 2000
Applicant's or agent's file reference 270142000340	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US98/23532	05 NOVEMBER 1998	05 NOVEMBER 1997

Applicant
BIOZONE LABORATORIES, INC.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	HELEN PRATT DEBORAH THOMAS PARALEGAL SPECIALIST
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Form PCT/IPEA/416 (July 1992)★

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 270142000340	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US98/23532	International filing date *(day/month/year)* 05 NOVEMBER 1998	Priority date *(day/month/year)* 05 NOVEMBER 1997
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant BIOZONE LABORATORIES, INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☒ Certain documents cited

   VII ☒ Certain defects in the international application

   VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04 JUNE 1999	12 FEBRUARY 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer HELEN PRATT DEBORAH THOMAS PARALEGAL SPECIALIST Telephone No. (703) 308-1978

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/23532

I. Basis of the report

1. This report has been drawn on the basis of *(Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments)*:

[X] the international application as originally filed.

[X] the description, pages 1-10 , as originally filed.
pages NONE , filed with the demand.
pages NONE , filed with the letter of _____.
pages _____ , filed with the letter of _____.

[X] the claims, Nos. 1-14 , as originally filed.
Nos. NONE , as amended under Article 19.
Nos. NONE , filed with the demand.
Nos. NONE , filed with the letter of _____.
Nos. _____ , filed with the letter of _____.

[X] the drawings, sheets/~~fig~~ none , as originally filed.
sheets/~~fig~~ NONE , filed with the demand.
sheets/~~fig~~ NONE , filed with the letter of _____.
sheets/~~fig~~ _____ , filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[X] the description, pages NONE.
[X] the claims, Nos. NONE.
[X] the drawings, sheets/~~fig~~ NONE.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the ~~Supplemental Box~~ Additional observations below (Rule 70.2(c)).

4. Additional observations, if necessary:

NONE

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/23532

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citati ns and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims	4-14	YES
	Claims	1-3	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-14	NO
Industrial Applicability (IA)	Claims	1-14	YES
	Claims	NONE	NO

2. CITATIONS AND EXPLANATIONS

Claims 1-3 lack novelty under PCT Article 33(2) as being anticipated by Melnik et al.

Melnik et al. disclose a composition such as infant milk which contains liposomes in amounts enough to enhance the bioavailability of ingredients and which deliver nutrients such as gamma linolenic acid as in claims 1-3 which contain glycerphospholipids in the claimed amounts (col. 4, lines 64-68 and col. 5, lines 1-68 and col. 6, lines 1-68).

Claims 4, 6-12 lack an inventive step under PCT Article 33(3) as being obvious over Melnik et al.

Claim 4 differs from the reference in the particular size of the liposomes. However, it is not seen at this time that the liposomes of the reference would not have the claimed size. It would have been within the skill of the ordinary worker to use a size capable with a baby formulation. Therefore, it would have been obvious to use a particular size of liposome.

Claim 6 further requires an emulsion of edible oils in an aqueous solution. Certainly, if liposomes are fatty materials and they are added to milk, and mixed an aqueous emulsion would have occurred. Therefore, it would have been obvious to make a formulation containing an aqueous emulsion.

Stabilizers such as carrageenan as in claim 7 are well known ingredients in milk formulations and nothing new or unobvious is seen in their use. Therefore, it would have been obvious to add known stabilizers for their known function to a liquid formulation.

Claim 8 requires that the bilayer forming lipids assemble into liposomes and act as emulsifiers to stabilize the solution. However, this is the way that liposomes are made and nothing new is seen in this process. Therefore, it would have been obvious (Continued on Supplemental Sheet.)

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication Date *(day/month/year)*	Filing Date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
US, A, 5,591,479	07 JANUARY 1997	29 JUNE 1995	NONE
US, A, 5,569,464	29 OCTOBER 1996	24 MAY 1995	02 APRIL 1993

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Claims 6, 10 and 13 are objected to under PCT Rule 66.2(a)(iii) as containing the following defect(s) in the form or contents thereof: "emulsions" should be "emulsion". Claim 10 should have a "period" at the end of the claim. Claim 13 (c), 2nd line "powder" should be "powdered".

VIII. Certain bservati ns n the internati nal application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 10 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): Claim 10 is indefinite in lacking antecedent basis for the term "phospholipid" in claim 1.

Supplemental B x
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:
The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(6): A23C 9/15, 9/158, 21/06; A23P 1/04 and US Cl.: 426/71, 72, 73, 98, 99, 641, 656, 582, 801

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
to use phospholipids to form bilayers in the claimed composition.
Claim 9 further requires additional nutrients, etc. Melnik et al. disclose a composition containing vitamins, protein and immuoglobulins and proteins (col. 7, lines 15-50).

Claim 10 differs from the reference in requiring that the formulation has the same concentration of phospholipid as in human milk. As the amount of phospholipid in human milk is known and it obviously agrees with infants, it would have been within the skill of the ordinary worker to copy the amount of phospholipids in human milk. Therefore, it would have been obvious to use a known amount of phospholipid in the claimed composition.

Claim 11 differs from the reference in the use of purified phospholipids entrapped by the liposomes. However, this is the normal way of making liposomes. Therefore, it would have been obvious to use phospholipids to entrap other ingredients.

The use of the various B vitamins are disclosed in col. 7, lines 15 -50 as is iron as in claim 12. It would have been obvious to use known forms of such in the claimed composition. Claim 5 lacks an inventive step under PCT Article 33(3) as being obvious over the prior art as applied in the immediately preceding paragraph and further in view of Endo et al.

Claim 5 differs from the above reference in the use of cholesterol in the liposome. Endo et al. disclose that it is known to use cholesterol or other sterols in forming liposomes in amounts from 0.05 to .5 parts (col. 2, lines 46-56). Therefore, it would have been obvious to use cholesterol in a liposome membrane composition.

Claims 13 and 14 lack an inventive step under PCT Article 33(3) as being obvious over the prior art as applied in the immediately preceding paragraph and further in view of Ponroy.

Claim 13 and 14 differ from the reference in the particular method of making the liposomes.

Claim 13 differs from the reference in the method of making an infant formula. However, the specification discloses on page 5, lines 11-25, that a method is known to encapsulate micronutrients, vitamins, immunoglobulins, and minerals into liposomes and that it is known to dehydrate the liposomes using known drying techniques. Ponroy discloses that it is known to incorporate cerebral phospholipids (nutrients) into milk for new born babies ((col. 4, lines 49-51). Melnik discloses as in claim 14 adding the liposomes to milk to make a liposome dispersion (col. 5, lines 50-56). Adding water to a powdered formula as in claim 14 is well known, and the liposomes would have inherently reformed. Therefore, it would have been obvious to add liposomes to a powdered infant formula and to rehydrate it.

Claims 1-14 meet the requirements for industrial applicability as defined by PCT Article 33(4) in providing a composition and process of making an infant milk formulation which contains liposomes in amounts to enhance nutritional deliery of nutrients.

---------- NEW CITATIONS ----------
US 5,591,479 A (PONROY) 07 January 1997, see abstract and col. ` lines 50-60, col. 4, lines 10-51).

US 5,569,464 A (ENDO et al) 29 October 1996, see abstract and col. 2, lines 45-55.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: ENHANCED INFANT FORMULA CONTAINING LIPOSOME ENCAPSULATED NUTRIENTS AND AGENTS

(57) Abstract

An infant formula contains liposomes which improve the nutritional delivery of nutrients, stabilize ingredients, and enhance their bioavailability. The formula more closely resembles the ultrastructure and infrastructure of natural human milk due to the presence of liposomes. The lipid concentration is in the range of 0.1–50 % of the formulation. The typical size of the liposomes range between about 20 nm and about 50 nm. The formula can be formulated to be in a liquid or dry form. The phospholipid concentration is the same as that which occurs in human milk.

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ENHANCED INFANT FORMULA CONTAINING LIPOSOME ENCAPSULATED NUTRIENTS AND AGENTS

Technical Field

This invention relates generally to the formulation of infant milk formula and more specifically to the composition and ultrastructure of infant formula to be more like mother's milk.

Background Art

Breast-feeding is, without question, the preferred method of feeding infants in the first months of life. The benefits of human milk both nutritional and nonnutritional have been thoroughly discussed (Fomon, S.J., Infant Nutrition, WB Saunders, Philadelphia, 1978, and Oski, F.A., in "Pediatric Nutrition," ed. F. Lifshitz, Marcel Dekker, New York, Ch. 3, pp. 55-62, 1980) in support of the belief that it is the optimal source of nutrition for the developing infant. Human milk provides essential quantities of energy, protein, carbohydrates, minerals and vitamins to achieve growth of the healthy infant. The nonnutritional benefits contribute to the well being of both mother and child. They include: developing the mother-child bond, breast fed infants have less childhood bacterial and viral infections; they have a reduced incidence of severe or obvious atopic disease, and are less susceptible to hypothyroidism. Maternal benefits include reduction of the incidence of breast cancer, and early repeat pregnancy.

Human milk has been well studied and reviewed over the last century (Pipes, P., Nutrition in Infancy and Childhood, 4th ed., St. Louis, Times Mirror/Mosby College Publishing, 1989, and Williams, A.F., Textbook of Pediatric Nutrition, 3rd ed. London: Churchill Livingston, 1991). Analysis of the composition of human milk reveals that it is an elaborate solution that contains more than 200 fat-soluble and water-soluble ingredients.

The concentration of nutrients in human milk has been used as the gold standard by which all forms and sources of infant nutrition are judged. Breast milk from a well nourished woman, if taken in adequate quantities by the infant, provides adequate daily requirements of minerals, vitamin A, thiamine, riboflavin, niacin, pyridoxine, vitamin B_{12}, folic acid, ascorbic acid, and vitamin E. The amounts of vitamin D, vitamin K and iron are often low and may require supplementation.

Lactose is the sole carbohydrate source in human milk. It is enzymatically broken down by lactase into galactose and glucose and absorbed through the small intestine. Milk proteins are defined broadly as either whey or casein. Casein is a mixture of phosphoproteins, rich in essential and common amino acids. Whey from human milk consists of alpha-lactoalbumin, lactoferrin, albumin, and immunoglobulins IgA, IgG, and IgM. The fat components of human milk contribute 3-4.5% of fat per 100 ml. The major fatty acids in human milk are stearic, oleic, plamitic and linoleic acids which provide the building blocks that form triacylglycerols (triglycerides) which make up 98-99% of the total fat in milk. In addition, phospholipids and cholesterol contribute 1-2% of total fat. (Hamosh, M., et al., Pediatrics (1985) 75(suppl):146-50.

The components and individual ingredients of human milk help make this nutritional substance the ideal food for infants. In addition, however, the ultrastructure of human milk is an essential factor in its biological performance. Some primary papers and review articles (Jensen, R.G., Progress in Lipid Res (1996) 35(1):53-92) deal with the microscopic ultrastructure of milk. The ultrastructure bodies that have been identified include: micelles, submicelles, fat globules, and milk fat globule membrane (MFGM, the proteinaceous coat surrounding fat globules). The complex milk protein system that makes up casein is known to form micelles and submicelles. Kappa-casein is the protein fraction of milk that allows formation of micelles and determines micelle size and function, thus affecting many of the physical characteristics of milk.

The milk fat globule is another complex body made up of triglycerides and the structure-function relationship is one of the factors controlling digestion. The

histochemistry and microscopic structure of human milk fat globule membrane is thoroughly treated by Buchheim, W., *et al.*, "Electron microscopy and carbohydrate histochemistry of human milk fat globule me.," in: Hansen, L.A., ed. Biology of human milk, Nestle Nutrition Workshop Series, Vol. 15, Raven Press, New York, 1988.

In many areas of the world, and in many situations, breast-feeding is not possible due to factors including mother-infant separation, infant inability or disease state, and mother inability or disease state. The nutrition of choice in these cases is infant formula. Commercially available infant formulas have been marketed since the early 1900s and have reached their current state of quality and evolution over the past 65 years. Advances in nutrition, biology and medicine during this time period have allowed infant formulas to achieve high nutritional quality, safety, and uniformity.

The aim of infant formulation is to make the very best substitute possible and to make the preparation more like mother's milk. Many existing formulas combine the same ingredients, have the same amount of calories, match renal solute load and achieve the exact osmolarity and osmolality as the standard, mother's milk. However, the complex ultrastructure of human milk has not been duplicated in infant formulas due to expense, technological know-how, and complete knowledge of ultrastructure.

This suggests that there is a need for new formulations that are chemically, calorically, compositionally, and nutritionally the same as human milk as well as structurally the same as human milk to meet the needs of developing infants worldwide.

Liposomes are microscopic lipid vesicles comprised of a lipid bilayer membrane that surrounds and separates a water compartment. A liposome can have a single bilayer membrane called a small unilamellar vesicle (SUV) or many layers which is called a multilamellar lipid vesicle (MLV). The membrane of liposomes is made from bilayer forming lipids, for example, phospholipids, sphingolipids, and cholesterol. Liposomes were first described by Banhem *et al.*, *J Mol Biol* (1965) 13:238-252. Liposome technology has evolved over the past 30 years to become a preeminent drug and nutritional delivery science. Liposomes have been used in

applications ranging from decreasing the cardiotoxicity of cancer drugs to topical penetration enhancement to gene delivery since their discovery.

Liposomes can encapsulate a variety of biologically active ingredients. The interaction of different molecules with liposomes such as water-soluble molecules are entrapped, or bound, either hydrophobically, electrostatically, or electrodynamically, to the liposome surface. Amphiphilic molecules orient into bilayers, and hydrophobic substances are dissolved in the bilayer. Complex macromolecules and proteins can also find different ways to accommodate and anchor into or bind or adsorb onto the bilayer. In particular cases some hydrophobic molecules can be entrapped or loaded into the liposome interior at so high concentrations that they precipitate or gel inside. Lasic, D.D., Liposomes: From Physics to Applications, Elsevier, New York, pp. 6-7, 1993.

Keller *et al.* have recently discovered the presence of liposomes in human milk. Electronmicrographs show the presence of SUVs and MLVs in the size range of 50-100 nm. these liposomes are thought to be comprised of the phospholipids, sphingomylens, and cholesterol, which exist in human milk. Because liposomes have also been shown to enhance the oral bioavailability of ingested ingredients (Maitani, Y. *et al.*, *J Pharm Sci* (1996) 85(4):440-445 and Sakuragawa, N. *et al.*, *Thrombosis Res* (1985) 38(6):681-685) that are poorly absorbed or not absorbed at all with liposome encapsulation, the use of liposomes orally has important applications such as in orally ingested products such as infant formulas. Since formula cannot match mother's milk in general availability of nutrients, the presence of liposomes may help explain this fact. This important ultrastructure discovery further characterizes human milk and makes possible formulating infant formula to be even closer to mother's own, and to enhance bioavailability of nutrients in a variety of orally consumed products.

Disclosure of the Invention

The present invention broadly relates to the use of liposomes in nutritional supplement products, drug products, and infant formula products for oral use in

mammals and to improve the nutritional delivery of nutrients, stabilize ingredients, and enhance the bioavailabilty of ingredients in these products using liposomes.

The materials used to form liposomes in this invention include any natural, bilayer forming lipids including those lipids from the classes of glycerolphospholipids, glyceroglycolipids, sphingophospholipids, and sphinogoglycolipids. The concentration of lipid used to form liposomes in this invention can range from 0.1-50% of the formulation. The resulting liposomes have a typical size range of 20nm-500nm. Cholesterol, or another sterol such as stigmasterol, can be added to the formulation to enhance the stability of the liposome membrane in concentrations of 0.05-30%.

Micronutrients, proteins, immunoglobulins, vitamins and mineral were encapsulated into liposomes using a modification of the reverse phase evaporation technique. (Lasic, DD. Liposomes. From physics to applications. Elsevier Press, New York. 1993; 92-94.) in order to: 1) prevent oxidation of ingredients, 2) stabilize the colloidal formulation, 3) enhance the oral bioavailability of encapsulated and associated nutrients, 4) sequester ingredients from one another to prevent interactions, and 5) increase stability of the encapsulated ingredients.

Enhancement of oral bioavailibility due to liposomes in the formulation, and in mothers milk, is predicated on the fact that polar lipids assist nutrient and fat absorption. Normally, when infant formula or mothers milk reaches the upper duodenum, where bile salts are secreted, micelles form to help assist in the dispersion and emulsification of fats and triglycerides. In the present invention, liposomes add another component to the mixture by contributing mixed vesicles. Polar lipids and bile salts form mixed micelles and mixed vesicles which increase absorption of fats and oil soluble ingredients in milk in the intestine.

Liquid infant formulations are emulsions of edible oils in an aqueous solution. Frequently infant formulas contain stabilizers, such as carrageenan. When bilayer forming lipids assemble into liposomes then also act as emulsufiers and stabilize the solution so carrageenan or other emulsifiers and stabilizers are not needed.

Another aspect of this invention is that the nutrients, vitamins, immunoglobulins and proteins can be encapsulated into liposomes and this complex can be dehydrated by known drying techniques and then combined with dry whey powder and other ingredients to make powder infant formula. When this powder

formula is added to water and stirred the liposomes will reform, the resultant solution is a liposomal dispersion.

Modes of Carrying Out the Invention

The following examples are intended to illustrate but not to limit the invention.

Example 1

Formula 1

Ingredient	Conc./L	% w/w
Purified Water		98.32%
Purified Lecithin (Phospholipon 90)		1.0%
Cis 4,7,10,13,16,19 Docosahexaenoic Acid (Sigma)	500 mg	0.05%
Arachidonic Acid (Fluka)	300 mg	0.03%
Vitamin E (Tocopheryl Acetate)		0.1%
Cholesterol (Sigma)		0.5%

Formula 2

Ingredient	Conc./L	% w/w
Purified Water		98.39%
Zinc (from Zinc Acetate)	10 mg	0.001%
Iron (from Ferrous Sulfate)	16 mg	0.0016%
Copper (from Cupric Sulfate)	0.8 mg	0.00008%
Selenium (from Sodium Selenate)	0.2 mg	0.00002%
Purified Lecithin (Phospholipon 90)		1.0%
Vitamin E (from Tocopheryl Acetate)		0.1%
Cholesterol (Sigma)		0.5%

Formula 3

Ingredient	Conc./100 ml	% w/w
Non-fat cow's milk		34.0%
Purified Water		21.0%
Formula 1		10.0%
Formula 2		10.0%
Lactose	4.55 g	4.55%
Palm Olein		7.0%
Soy Oil		6.0%
Sunflower Oil		7.0%
Vitamin A	200 IU	0.00011%
Vitamin D	40 IU	$1x10^{-9}$%
Vitamin E	1.5 IU	0.0015%
Vitamin K	6.0 mcg	$6x10^{-6}$%
Thiamine	40.0 mcg	0.00004%
Riboflavin	100.0 mcg	0.0001%
Vitamin B6	50.0 mcg	0.00005%
Vitamin B12	0.22 mcg	$2.2x10^{-7}$%
Niacin	500.0 mcg	0.0005%
Folic Acid	6.0 mcg	$6x10^{-6}$%
Pantothenic Acid	300.0 mcg	0.0003%
Ascorbic Acid	6.0 mg	0.006%
Biotin	1.2 mcg	$1.2x10^{-6}$%
Choline	12.0 mg	0.012%
Inositol	15.0 mg	0.015%
Calcium	50.0 mg	0.05%
Phosphorus	36.0 mg	0.035%
Magnesium	5.0 mg	0.005%
Manganese	5.0 mg	0.005%
Iodine	6.0 mg	0.006%
Sodium	10.0 mg	0.01%
Potassium	60.0 mg	0.06%
Chloride	20.0 mg	0.02%

In this example, a milk-based infant formula (Formula 1, 2 or 3) is prepared with the same concentration of phospholipid that occurs in human milk. Using purified phospholipids from soy (Phospholipon 90H, Natterman Phospholipid, Cologne, Germany), liposomes were formulated which entrapped zinc, iron, copper, and selenium, into one liposome system and docosahexenoic acid (DHA), arachidonic

acid were entrapped into another liposome system. The purpose of this formulation was to sequester the respective encapsulates and prevent interaction in the final formulation where the minerals can cause the oxidation of the lipids.

Example 2

Formula 1

Ingredient	% w/w
Purified Water	51.8%
L-Carnitine HCL (Sigma)	20.0
Purified Lecithin (Phospholipon 90H)	2.0%
Cholesterol (Sigma)	1.0%
Tocopheryl Acetate	0.2%
Palm Olein	10.0%
Fructose	10.0%
Lactose	5.0%

In this example, L-carnitine was encapsulated into a liposome using purified phospholipids from soy (Phospholipon 90H) and add liposome/L-carnitine to a milk-based infant formula. L-carnitine has poor oral bioavailability. The purpose of this formulation was to enhance the oral bioavailability of L-carnitine.

Example 3

Formula 1

Ingredient	Conc./L	% w/w
Purified Water		81.999%
IgG Human (Fluka)	10.0 mg	0.001%
Purified Lecithin (Phospholipon 90H)		2.0%
Cholesterol (Sigma)		1.0%
Fructose		10.0%
Lactose		5.0%

In this example, three immunoglobulins, IgG, IgA, and IgE, were encapsulated. The purpose of this formulation was to stabilize these immunoglobulins in the infant milk-based product. In addition, by encapsulating them into a liposome that is made to withstand the hostile environment of the stomach they are delivered to the small intestine where they increase immunity of the infant.

Example 4

Formula 1

Ingredient	Conc./L	% w/w
Purified Water		91.125%
L-Arginine HCl	4.0 g	0.4%
L-Cystine HCl	2.3 g	0.23%
Taurine	450.0 mg	0.045%
Tocopheryl Acetate		0.2%
Purified Lecithin (Phospholipon 80H)		2.0%
Cholesterol (Sigma)		1.0%
Lactose		5.0%

In this example, arginine, taruine, and cystine were encapsulated into liposomes to enhance survival in the stomach and to enhance the oral bioavailability for these three amino acids.

Example 5

Ingredient	% w/w	
Purified Water	77.176	
Ascorbic Acid	0.3	
Citric Acid	0.3	
Dipotassium Hydrogen Phosphate (Mollinckrodt)	0.2	
Sodium Sulfate (Spectrum)	0.2	
Thiamine HCL, USP (Spectrum)	0.024	
Ferrous Sulfate (Spectrum)	1.8	
Hydrogenated Lecithin		20.0

In this example, thiamine HCl and ferrous sulfate were encapsulated into liposomes to enhance survival in the stomach and to enhance the oral bioavailability.

Claims

1. In an infant milk formulation wherein the improvement comprises liposomes in amounts to enhance nutritional delivery of nutrients, stabilize ingredients, and enhance the bioavailabilty of ingredients.

2. The infant formulation of claim 1 wherein liposomes include natural, bilayer forming lipids selected from glycerolphospholipids, glyceroglycolipids, sphingophospholipids, sphinogoglycolipids or mixtures thereof.

3. The infant formulation of claim 1 wherein the lipid concentration are in the range of 0.1-50% of the formulation.

4. The infant formulation of claim 1 wherein the liposomes have a typical size range between about 20nm and about 500nm.

5. The infant formulation of claim 1 wherein the liposome additionally include in concentrations of 0.05-30% cholesterol, stigmasterol or mixtures thereof to enhance the stability of the liposome membrane.

6. The infant formulation of claim 1 is an emulsions of edible oils in an aqueous solution.

7. The infant formulation of claim 1 additionally contains stabilizers, such as carrageenan.

8. The infant formulation of claim 6 wherein bilayer forming lipids assemble into liposomes which act as emulsufiers and stabilize the solution in the absence of carrageenan or other emulsifiers.

9. The infant formulation of claim 1 additionally includes nutrients, vitamins, immunoglobulins and proteins.

10. The infant formulation of claim 1 has the same concentration of phospholipid that occurs in human milk

11. The infant formulation of claim 1 has purified phospholipids from soy (Phospholipon 90H, Natterman Phospholipid, Cologne, Germany) the liposomes entrap thereby sequestering the respective encapsulates and preventing oxidation of the lipids.

12. The infant formulation of claim 1 wherein the nutrients are thiamine HCl and ferrous sulfate.

13. A process for preparing infant formula comprising,

a) encapsulating nutrients, vitamins, immunoglobulins, proteins or mixtures thereof into liposomes,

b) dehydrating the liposomes,

c) combining the dehydrated liposomes with dry whey powder and other ingredients to make powder infant formula.

14. A process for preparing infant formula of claim 13 further comprising, adding the powdered formula to water and stirring under conditions wherein the liposomes reform forming a liposomal dispersion.

INTERNATIONAL SEARCH REPORT

International application N .
PCT/US98/23532

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A23C 9/15, 9/158, 21/06; A23P 1/04
US CL :426/71, 72, 73, 98, 99, 641, 656, 582, 801

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 426/71, 72, 73, 98, 99, 641, 656, 582, 801

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS search terms: liposomes, milk, glycerolphospholipids, or glyceroglycolipids, encapsulate, infant, baby, thiamine, ferrous

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,591,446 A (MELNIK et al.) 07 January 1997, abstract and col. 5, lines 57-65, col. 6, lines 41-50.	1-11, 13, 14
X,P	US 5,707,670 A (MEHANSHO et al.) 13 January 1998, abstract and col. 5, lines 65-67 and col. 6, lines 1-5, lines 55-70.	1, 12
Y	US 5,013,569 A (RUBIN) 07 May 1991, abstract.	1
Y	US 4,255,454 A (BRANNER-JORGENSEN) 10 March 1981, col. 1, lines 25-40.	13
Y	US 4,497,836 A (MARQUARDT et al.) 05 February 1985, abstract, and col. 4, lines 5-15.	13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

07 JANUARY 1999

Date of mailing of the international search report

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